

## Fathead Minnow and Pearl Dace Pilot at Canadian Experimental Lakes Area

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### Science Question(s)

Determine how and to what degree human and wildlife populations are exposed to endocrine-disrupting compounds (EDCs). Determine what effects are occurring in exposed human and wildlife populations. What are the effects of long-term exposure to 17 $\beta$ -ethynylestradiol (EE2) in an aquatic ecosystem? What are the relationships of vitellogenin (VTG) gene expression in indigenous *Pimephales promelas* fathead minnows and pearl dace to biomarkers and population effects? What are the relationships of changes in indigenous gene expression to those in laboratory-cultured fathead minnows exposed to water and sediments from dosed lakes and control lakes? Can fathead minnow primers be used on pearl dace to detect changes in gene expression?

### Research

There is increasing concern about the potential impact of EDCs on aquatic organisms. Among the EDCs found in aquatic habitats are synthetic estrogens, which are used in contraceptives and other pharmaceuticals. These chemicals enter waterways through sewage treatment plants and septic systems and are also found in surface waters. EE2 is one of the most commonly used synthetic estrogens. Several studies have found EE2 in waters downstream of sewage treatment plants. In these rivers, concentrations of EE2 in the low ng/L levels are common, with some sites having concentrations as high as 40-60 ng/L.

A whole-lake endocrine-disruption experiment was conducted by Fisheries and Oceans Canada at the Experimental Lakes Area (ELA) in northwestern Ontario for three years beginning in 2001. This experiment examined population, organismal, biochemical, and cellular-level effects in lake trout, white sucker, fathead minnow, and pearl dace exposed to environmentally relevant (4–6 ng/L) concentrations of the synthetic estrogen, EE2. The U.S. Environmental Protection Agency (U.S. EPA) collaborated in this study by evaluating VTG gene expression in (1) indigenous fathead minnows and pearl dace collected from the dosed and control lakes in 2001 through 2003, before and after dosing; (2) indigenous minnows collected in 2001 from the control lake and deployed for 1, 3, 7, and 13 days in the dosed and control lakes; and (3) Cincinnati-cultured minnows exposed to water collected in 2001 through 2004 from ELA lakes and shipped to Cincinnati. In addition to water exposures, embryo–larval fish and adult male fathead

minnows were exposed to control- and dosed-lake whole sediment and sediment elutriate samples.

Methods developed to measure VTG gene expression in fathead minnows were validated for use in another cyprinid, pearl dace. Indigenous male fathead minnows and pearl dace collected at all time intervals from the dosed lake showed a constant level of elevation in VTG gene expression. Gene expression in the 2001 fathead minnow deployment study was detected within 24 hours after deployment of control fish into the treated lake and stayed elevated for the entire 13-day study. Highly variable gene expression was found in fathead minnow fry exposed to dosed-lake sediment elutriates, but no significant gene expression was found in fry exposed to control-lake sediment elutriates. Male adult fathead minnows exposed to sediment elutriates from sediments collected in 2004 in the previously dosed lake showed significant VTG gene expression. Results indicate that Real Time Polymerase Chain Reaction (RT-PCR) analyses of total RNA can be used to provide a rapid and timely estimate of exposure to estrogenic substances to indigenous organisms and in laboratory animals exposed to water and sediment samples. Furthermore, gene expression results, in combination with biochemical, histological, and chemical information, show that indigenous fathead minnows and pearl dace continuously exposed to 5 ng/L of EE2 will have adverse exposures.

### **Impacts and Outcomes**

An EDC multiyear plan, “Annual Performance Measure,” has been prepared and will be distributed to the Office of Water and regional offices to support the development of environmental policy and water quality criteria and standards development. A manuscript will also be prepared and submitted. Overall, this pilot research activity extended the capabilities of the U.S. EPA/ORD EDC monitoring efforts to a successful collaboration on an international whole-lake ecosystem study at an extremely remote location.

Although this work was reviewed by the U.S. Environmental Protection Agency and approved for publication, it may not necessarily reflect official Agency policy.